



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁴ : C12P 19/04, C08B 37/04 C12N 11/10 // (C12P 19/04 C12R 1:065)</p>	<p>A1</p>	<p>(11) International Publication Number: WO 86/ 03781 (43) International Publication Date: 3 July 1986 (03.07.86)</p>
<p>(21) International Application Number: PCT/NO85/00080 (22) International Filing Date: 16 December 1985 (16.12.85) (31) Priority Application Number: 845059 (32) Priority Date: 17 December 1984 (17.12.84) (33) Priority Country: NO</p> <p>(71) Applicant (for all designated States except US): SINTEF [NO/NO]; N-7034 Trondheim - NTH (NO).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only) : LARSEN, Bjørn [NO/NO]; Egganveien 16, N-7081 Sjetnhaugen (NO). BRÆK, Gudmund Skjåk [NO/NO]; Nedre Bergsving- en 6, N-7000 Trondheim (NO).</p> <p>(74) Agent: GRÆSBØLL, Sigrun, E.; Bryn & Aarflot A/S, P.O. Box 1364 Vika, N-0114 Oslo 1 (NO).</p>		<p>(81) Designated States: AT (European patent), BE (Euro- pean patent), CH (European patent), DE (European patent), FR (European patent), GB (European pa- tent), IT (European patent), LU (European patent), NL (European patent), SE (European patent), US.</p> <p>Published <i>With international search report.</i></p>
<p>(54) Title: PROCESS FOR PRODUCING ALGINATES HAVING IMPROVED PHYSICAL PROPERTIES, AND THE USE OF SAID ALGINATES</p>		
<p>(57) Abstract</p> <p>Process for producing alginates having improved physical properties, by the inoculation of alginates derived from brown algae or bacteria, with an enzyme preparation such as a mannuronan-C-5-epimerase preparation from <i>Azotobacter vinelandii</i>. The modified alginates are used for immobilizing enzymes, cell organelles or cells as well as for the microencapsulation of biocatalysts.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GA	Gabon	MR	Mauritania
AU	Australia	GB	United Kingdom	MW	Malawi
BB	Barbados	HU	Hungary	NL	Netherlands
BE	Belgium	IT	Italy	NO	Norway
BG	Bulgaria	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	LI	Liechtenstein	SN	Senegal
CH	Switzerland	LK	Sri Lanka	SU	Soviet Union
CM	Cameroon	LU	Luxembourg	TD	Chad
DE	Germany, Federal Republic of	MC	Monaco	TG	Togo
DK	Denmark	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali		
FR	France				

Process for producing alginates having improved physical properties, and the use of said alginates.

The present invention relates to the preparation of alginates having improved physical properties, especially with respect to the formation of gels having inorganic or polyvalent organic ions. Said modified alginates are intended to be used for immobilizing and encapsulating enzymes and/or cells for use in biotechnological processes.

Using alginate gels as an immobilizing material has several deficiencies, two of them being:

1) Calcium alginate gels are destabilized by compounds having affinity for calcium, for instance EDTA, citrate, lactate and phosphate, as well as high concentrations of cations such as Na^+ , K^+ and Mg^{++} ;

2) Alginates currently used have a high degree of chemical heterogeneity and provide gels having pores of such great size that proteins - enzymes and other macromolecules - can leach out, at the same time as the size distribution of the pores is difficult to control.

Alginate is the most important structural polysaccharide in marine brown algae and is used for several industrial purposes wherein the properties of the polymer are utilized as a polyelectrolyte - for instance for gel formation and thickening purposes - and also for its water and ion binding capacity.

The purpose of the present invention, thus, is to prepare alginates having physical properties satisfying the requirements for increased gel strength and stability and better controllable pore size.

Chemically seen, alginate is a polyuronide built up from two uronic acids, viz., D-mannuronic acid (M) and the C-5-epimer L-guluronic acid (G). They are arranged in such fashion that the polymer is further built up from three types of sequence: (G)-rich sequences called G-blocks, (M)-rich sequences called M-blocks and alternating structure symbolized by (MGMGMG).

The alginate's ability to form a gel by ionic binding, and the properties of said gel depends both on the relative content of the two uronic acids and on the distribution of the guluronic acid units along the chain.

A high content of (G)-blocks yields, for instance, an alginate with great gel-forming capacity, which, technically seen, is a valuable property of the polymer.

The present invention is based on the following:

The alginate is synthesized in the alga as polymannuronic acid and is thereafter modified by an enzyme, mannuronan-C-5epimerase, which converts D-mannuronic acid residues into L-guluronic acid residues within the chain.

When said enzyme affects the alginate, both the relative content and the uronic acid sequence will be changed and, consequently, its physical properties.

Thus, the invention relates to a process for producing alginates having improved physical properties such as increased gel strength, by using enzymatic modification on a polymeric level. The process is characterized in that alginates derived from brown algae or bacteria are inoculated with an enzyme preparation.

As such an enzyme preparation is preferably used a C-5-epimerase preparation, more preferably an alginate lyase-free mannuronan-C-5-epimerase produced from the earth bacterium Azotobacter vinelandii.

The present invention also comprises the use of the thus modified alginates for immobilizing enzymes, cell organelles and cells by entrapment in gels of alginate or alginate having suitable cations, as well as by immobilizing biocatalysts by encapsulation in alginate polycation microcapsules.

The mannuronan-C-5-epimerase may be isolated from cultures from the earth bacterium Azotobacter vinelandii, which produces both alginate and epimerase extracellularly. The fact that the enzyme is extracellular is a great advantage in the isolation process, and it also indicates that the enzyme may function freely in solution

independent on intracellular factors, which is favourable to a technical exploitation of the invention.

The use of immobilized enzymes as catalysts has obtained still greater importance in industry and will, in the years to come, become one of the most important expansion areas for biotechnology. Immobilized enzymes are often more stable, but first and foremost, they are easier to handle than free, soluble enzymes and may be used in continuous processes.

In addition to immobilizing simple enzymes there has also been developed techniques for immobilizing whole cells. The cells may serve as carriers for a single enzyme, such that isolation of the enzyme is unnecessary before immobilizing, or several enzymes may also be used in the cell in order to catalyse multistep processes (for instance synthesis of hormones, proteins, etc.).

We have tried out the epimerizing of a plurality of high polymer alga and bacterium alginates having varying block structures and formulation, and the conclusions are that all of the alginates can be epimerized to a substantial degree. The epimerization degree varies from 60 to 90 percent depending on the original block structure of the alginates and for some alginates this yields more than a doubling of the gel strength measured in 2 percent homogenous Ca-alginate gels.

Examples

Example 1

Sodium alginate derived from Laminaria digitata, in an amount of 0.07% by weight, was dissolved in cationic buffer, 0.05M collidine pH 7.0 and Ca^{2+} 6.8mM. This was incubated with a lyase-free C-5-epimerase preparation from A. vinelandii at 30°C for 8 hours. The epimerization degree, measured by means of high solution n.m.r.-spectroscopy, shows an increase of the guluronic acid content from 41% to 69% (see the Table).

Example 2

Sodium alginate from Macrocystis pyrifera, in an amount of 0.07% by weight, was dissolved in cationic buffer, 0.05M collidine pH 7.0 and Ca^{2+} 6.8mM. This was incubated with a lyase-free C-5-epimerase preparation from A. vinelandii at 30°C for 8 hours. The epimerization degree, measured by means of high solution n.m.r.-spectroscopy, shows an increase of the guluronic acid content from 37% to 62% (see the Table).

Example 3

Sodium alginate from Laminaria hyperborea, in an amount of 0.07% by weight was dissolved in a cationic buffer, 0.05M collidine pH 7.0 and Ca^{2+} 6.8mM. This was incubated with a lyase-free C-5-epimerase preparation from A. vinelandii at 30°C for 8 hours. The epimerization degree, measured by means of high solution n.m.r.-spectroscopy, shows an increase of the guluronic acid content from 68% to 79% (see the Table).

Example 4

Sodium alginate from Laminaria digitata containing 40% guluronic acid is treated with C-5-epimerase from A. vinelandii at pH 7.0 and Ca^{2+} 0.68mM for 6 hours at 30°C. The modified alginate contains 63% guluronic acid. Gel strength measurements on homogenous 2% calcium gels show a gel strength of 3.8 N/cm² and 9.6 N/cm² in native and enzyme modified alginate, respectively (see Figure 1).

TABLE

Composition and GG content of the polymer before and after enzymatic modification measured by high solution ^1H -n.m.r.-spectroscopy

Source	Before epimerization			After epimerization		
	F_G	F_M	F_{GG}	F_G	F_M	F_{GG}
<u>Laminaria digitata</u>	0.41	0.59	0.25	0.69	0.31	0.54
<u>Laminaria hyperborea</u>	0.68	0.32	0.57	0.79	0.21	0.67
<u>Macrocystis pyrifera</u>	0.37	0.63	0.14	0.62	0.38	0.32
<u>Elachistae sp.</u>	0.68	0.32	0.64	0.89	0.11	0.85
<u>Dichtiosyphon foenicula.</u>	0.67	0.33	0.61	0.81	0.19	0.75
<u>Ascophyllum nodosum</u>	0.36	0.64	0.16	0.63	0.37	0.39
<u>Azotobacter vinelandii</u> deacetylated	0.45	0.55	0.41	0.69	0.33	0.54

Claims

1. A process for producing alginates having improved physical properties, for instance increased gel strength, comprising inoculating alginates derived from brown algae or bacteria with an enzyme preparation.
2. The process of claim 1, wherein said enzyme preparation is a C-5-epimerase preparation.
3. The process of claim 1 or 2, wherein said mannuronan-C-5epimerase preparation is derived from Azotobacter vinelandii.
4. The use of alginates modified by the process according to any one of the preceding claims, for immobilizing enzymes, cell organelles and cells by gel entrapment in alginate gels or microcapsules of alginate and poly-cations.

1/1

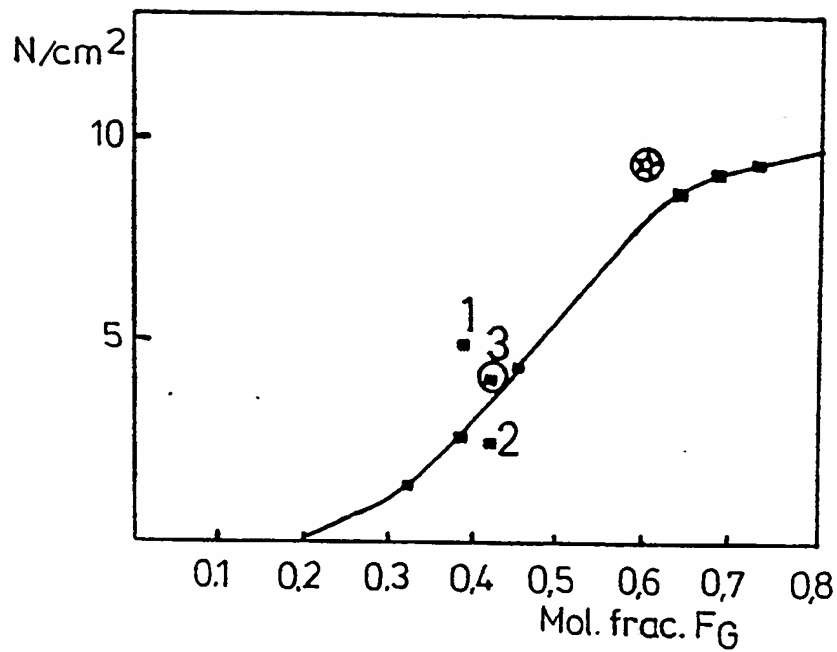



Fig.1

Gel strength measured on 2% alginate gels as a function of the guluronic acid content. 1. Macrocystis; 2. Acophyllum; 3. Laminaria digitata; ⊗ Enzyme treated with L. digitata.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/NO85/00080

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC 4 C 12 P 19/04, C 08 B 37/04, C 12 N 11/10 // (C 12 P 19/04, C 12 R 1:065)		
II. FIELDS SEARCHED		
Minimum Documentation Searched 7		
Classification System	Classification Symbols	
IPC 4	C 12 P 19/04; C 08 B 37/04; C 12 R 1:065; C 12 N 11/04; C 12 N 11/10	
US Cl	536:3; 435:101; 195:31	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *		
SE, NO, DK, FI classes as above		
III. DOCUMENTS CONSIDERED TO BE RELEVANT *		
Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X, Y	Chemical abstracts, Vol 75, (1971) abstract No 564k, Carbohyd Res, 1971, 17(2), 297-308 (Eng.)	1-3, 4
Y	Derwents abstract No 13334 D/08, SU 742 434	4
Y	Patent abstracts of JP 59-74984, published 1984-04-27	4
X, Y	Chemical abstracts, Vol 76, (1972), abstract No 31806r, Carbohyd Res 1971, 20(2), 225-32 (Eng).	1-3, 4
X, Y	Chemical abstracts, Vol 72, (1970), abstract No 75879p, Biochim. Biophys. Acta 1969, 192(3), 557-9 (Eng).	1-3, 4
X, Y	Chemical abstracts, Vol 101, 1984, abstract No 166114u, Gums. Stab. Food. Ind. Appl. Hydro-colloids, Proc. Int. Conf. 2 nd 1983, (publ. 1984), 523-528 (Eng). .../...	1-3, 4
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: 10</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
1986-04-04	1986 -04- 08	
International Searching Authority	Signature of Authorized Officer	
Swedish Patent Office	 Yvonne Siösteen	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X, Y	Chemical abstracts, Vol 82, 1975, abstract No 167310f, Proc. Int. Seaweed Symp 7 th 1971, (Publ 1972), 491-495 (Eng):	1-3, 4
A	Chemical abstracts, Vol 95, 1981, abstract No 147178j, Nippon Suisan Gakkaishi 1981, 47(7), 889-93 (Eng).	1-4